PRELIMINARY REMARKS ON PHOSPHORESCENT BACTERIA FROM SEA-WATER.

By Dr. Oscar Katz.

Influenced by a memoir recently published by Dr. Fischer, on a light-producing bacterium found in sea-water near the Danish Island of St. Croix, in the West Indies (1), and also by his statements on another kind of fission-fungus derived from dead marine fish out of the Baltic Sea and the Berlin Aquarium (2), I commenced to look for phosphorescent schizomycetes which might occur in the sea-water of our vicinity (Sydney). My endeavours have hitherto proved so far successful that up to now I have been able to obtain three kinds of this very interesting group of micro-organisms, which are capable of cultivation in various nutritive substances, which can be transferred to marine animals (fish, crustaceans), so as to show what often happens spontaneously (so-called self-phosphorescence of fishes, &c.), and which on being added to common sea-water are able to render this luminous in such a way that it produces an effect similar to certain kinds of what is known under the general name of phosphorescence of sea-water.

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(1) "Bacteriologische Untersuchungen auf einer Reise nach Westindien" von Dr. Fischer, Marinestabsarzt. II. "Ueber einen lichtentwickelnden in Meerwasser gefundenen Spaltpilz, *Zeitschrift f. Hygiene*, Bd. II., Heft 1, Leipzig, 1887, pp. 54-92.

(2) Addendum to the above publication, pp. 92-95. A paper by Dr. O. Hermes on, as I must believe, the same bacterial species, which he has named *Bacterium phospherescens*, I have not yet seen. A short note of it is given in "Nature," February 17, 1887, p. 377.

I.

The first kind appertaining to the above group of bacteria was derived by me indirectly from sea-water, inasmuch as I obtained it from dead marine fish, which were procured fresh at the Sydney Fish Markets, and which after some time became luminous by themselves. From sea-water itself I have not succeeded yet in cultivating it; its regular appearance on various marine fish which are being kept moist and at a moderate temperature, goes to show that its *habitat* is sea-water.

This microbe to which I have given the name of *Bacillus smaragdino-phosphorescens*, forms, in its adult state, short thick rods of about ·001 mm. width, and is about double as long as wide.

The extremities are rounded off. It is not motile and does not show filaments so far as I could see. After treatment with aniline dyes the bacilli are very distinctly seen to be stained only at their peripheral parts, while a central spot, similar to a "vacuole," remains unstained.

They grow on and in nutrient gelatine without liquefying it. Full particulars relative to their mode of growth will be given at another time, here I may state that they spread themselves on the gelatine but little, the ultimate size of their colonies being not very considerable.

The temperature at which this micro-organism develops best is about 20° C. (68° F.), or a little higher, and it is then that the light which its cultures emit is strongest. The colour of this light is a wonderful emerald green.

At temperatures between 13° C. and 15° C. ($55\frac{2}{5}$°-59° F.) the bacillus grows rather slowly, and the emitted light is then less conspicuous and intense than that of cultures kept at the above temperatures.

Whether this bacterial species is identical with that described by Dr. Fischer (l.c., pp. 92-95), and the *Bacterium phosphorescens* of Dr. Hermes (l.c.), is still doubtful; a satisfactory answer can be arrived at when more information in consequence of continued observations shall be available.

II.

The second kind of phosphorescent bacteria, to be named *Bacillus argenteo-phosphorescens*, was repeatedly obtained by me from sea-water at Elizabeth Bay, Port Jackson, Sydney. On gelatine, after having been mixed with 10 drops of this water, there would appear, among a considerable number of other colonies, an average number of no more than two luminous colonies which belonged to the above-named species.

Under high powers of the microscope it exhibits slender rods, which are tapering at their extremities and commonly slightly curved. In their adult state they measure about ·0025 mm. in length, and are about three times as long as broad. They are motile, and form, so far as I can judge, no filaments.

For alkaline methylene-blue they seem to have little affinity; for they do not take up this dye so eagerly as is done by most bacteria. Aniline-fuchsin or aniline-gentian-violet yield better results.

On and in nutrient gelatine they develop to characteristic colonies of which a detailed description will be given shortly. They do not liquefy the gelatine, and spread themselves on it far more than can be noticed in the case of Bacillus No. I.

Bacillus argenteo-phosphorescens grows best at temperatures of from 14° to about 23° C., and between these limits there is also the *optimum* of its luminosity, this *optimum*, however, inclining rather to the lower than to the upper of these temperatures. The light, emitted by its cultures in the dark, is of a mild, silvery appearance, and less brilliant than that of *Bacillus smaragdino-phosphorescens* and of the following one.

III.

The third kind of bacteria alluded to, which I propose to name *Bacillus cyaneo-phosphorescens*, I obtained, on the 6th of this month (June), from sea-water at Little Bay, 10 miles to the south of Sydney. In a tube of nutritive gelatine mixed with 10 drops of this water, and solidified after the manner of Esmarch, I noticed a few days afterwards, besides a good many other colonies, two luminous ones, which were made up of the above bacillus.

This kind is represented by straight rods, measuring about ·0026 mm. in length, and being about $2\frac{1}{2}$ times as long as broad. They are rounded off at their extremities; they show spontaneous movements, and are often found as diplobacillus, not so often in chains. These are commonly bent, attaining here and there a considerable length. With alkaline methylene-blue they stain fairly well, but a small central portion of them remains unstained. Yet this appearance is not so striking as in *Bacillus smaragdino-phosphorescens*, which shows the differentiation between a well-coloured peripheral and an uncoloured inner part in a very characteristic manner.

Bacillus cyaneo-phosphorescens grows rather slowly on and in nutritive gelatine which gradually becomes liquefied by it. In this regard it differs widely from the two other kinds which, as mentioned, cause no liquefaction of the gelatine. It thrives far better on nutrient agar-agar, where after a comparatively short time, it forms a substantial, greyish-white, sticky layer.

The *optimum* of growth as well as of luminousness for this microbe is between 20° C. and 30° C.; a temperature fluctuating between 13° and 15° C., however, does not seem at all unfavourable to its propagation or deleterious to its power of luminosity, although higher temperatures as above intensify both growth and phosphorescence. The colour of the light emitted in the dark or at least in sufficiently dark surroundings is of a decidedly bluish tint, and seems to stand, as regards its degree, between those of Bacillus No. I. and No. II.

Comparing Dr. Fischer's description of the West Indian *Bacillus phosphorescens* with what I have already ascertained about the bacillus from Little Bay, I am almost inclined to consider these two organisms as identical. However, I hesitate to pronounce a definite opinion until I have made a larger number of individual observations.

In giving, as has been done above, a few preliminary remarks on these three kinds of light-producing bacteria from sea-water—a

more elaborate paper on this subject I am going to prepare for a future Meeting—I wish to call attention to this interesting subject, as the question of the cause of certain kinds of phosphorescence of sea-water, for the explanation of which nothing certain as yet has been advanced, will now, it is to be hoped, soon be solved. Pflüger (quoted from Dr. Fischer's Treatise, l.c., p. 55), already suggested, a number of years ago, that micro-organisms of the group *Bacteria* participate in the production of phosphorescence of sea-water, and the experiments recently made by Fischer with pure cultures of luminous schizomycetes on ordinary sea-water, convinced him of the striking resemblance which an artificially produced luminosity of sea-water bears to that magnificent phenomenon described by English writers as "milky sea." The direct proof, he says, that such an appearance is brought about by bacteria of the above nature, is still a desideratum, but by means of continued researches it is sure to succeed. For my part I have not the least doubt that this will be the case, to judge from what I have read and heard about "milky seas"—I have not yet been fortunate enough to come across such a phenomenon—and from experiments made by me on sea-water with pure cultures of the three species of bacteria mentioned. A systematic or occasional search for such like sea-water bacteria at different places of the globe, may no doubt add to the number of kinds already found, although I believe the number of them will not become very large. Those forms which are now known belong to the aërobic class of micro-organisms, that is to say, they neither grow nor emit light without the presence of air (oxygen). Whether or not phosphorescent bacteria of the anaërobic class, propagating only with the exclusion of oxygen, may be detected in sea-water, either directly or indirectly (in marine animals), and whether or not such micro-organisms may play a part in certain kinds of phosphoresence of sea-water, all this is still an open question. There is on record the statement by two investigators, Bancel and Husson, (1)

(1) Sur la phosphorescence de la viande de homard. *Comptes rendus*, 1879, Vol. 88, pp. 191-192.

namely, that besides an aërobic form at the mucous surface of luminous lobster-flesh, they found inside this mucus an anaërobic one of extremely small dimensions, a micro-organism which, they say, produces carburetted and phosphoretted hydrogen, by the combustion of which phosphorescence is produced. Then Lassar (1) suggested the idea that perhaps the phosphorescence of some of the numerous phosphorescent marine animals might be brought about by parasitic micro-organisms. It is after all not impossible that anaëorbic forms may be found to be the cause of the luminosity of a number of luminous marine animals, which would then contribute only mediately to the phosphorescence of sea-water.

(1) Quoted from Fischer, l.c., p. 92.